Percutaneous malathion absorption by anuran skin in flow-through diffusion cells

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Abstract

There is increased concern about the sublethal effects of organophosphorous (OP) compounds on human and animal health, including the potential role of OP compounds in the global decline of amphibian populations. Malathion is one of the most widely used OP pesticides with numerous agricultural and therapeutic applications, and exposure to environmentally applied malathion can lead to adverse systemic effects in anurans. Cutaneous absorption is considered a potentially important route of environmental exposure to OP compounds for amphibians, especially in aquatic environments. One *in vitro* system commonly used to determine the absorption kinetics of xenobiotics across the skin is the two-compartment Teflon flow-through diffusion cell system. To establish cutaneous absorption kinetics of malathion, six full thickness skin samples taken from both the dorsal and ventral surfaces of each of three bullfrogs (*Rana catesbeiana*) and three marine toads (*Bufo marinus*) were placed into two-compartment Teflon flow-through diffusion cells perfused with modified amphibian Ringer's solution. A 26 μg/cm^2 dose of malathion-2,3-^{14}C diluted in 100% ethanol was applied to each sample (0.44–0.45 μCi). Perfusate was collected at intervals over a 6 h period and analyzed for ^{14}C in a scintillation counter. At the end of 6 h, surface swabs, tape strips, biopsy punches of the dosed area of skin, and peripheral samples were oxidized and analyzed for residue effects. Malathion absorption was greater across the ventral skin compared to dorsal skin in both bullfrogs and marine toads.
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Keywords: Organophosphorous; Pesticide; Bullfrog; *Rana catesbeiana*; Marine toad; *Bufo marinus*

1. Introduction

There is increased concern about the sublethal effects of organophosphorous (OP) compounds on human and animal health (Marty et al., 1994; Douglas, 1995; Gralewicz and Socko, 1997; Azaroff, 1999; Sheffield and Lochmiller, 2001; Wood and Stark, 2002; Jaga and Dharmani, 2003), including the potential role of OP compounds in the global decline of amphibian populations (Schuytema et al., 1993; Taylor et al., 1999a,b; Fordham et al., 2001; Kiesecker, 2002; Gilbertson et al., 2003; Relyea, 2004). Malathion is one of the most widely used OP pesticides with numerous agricultural and therapeutic applications (Marty et al., 1994; Gralewicz and Socko, 1997; Walker and Johnstone, 2000; Sheffield and Lochmiller, 2001; Zhang et al., 2002; Hunter and Barker, 2003). Exposure to environmentally applied malathion can lead to adverse systemic effects in anurans, including effects on the nervous system and behavior (Gralewicz and Socko, 1997; Relyea, 2004), development (Fordham et al., 2001; Kiesecker, 2002), the reproductive system (Sheffield and Lochmiller, 2001), and the immune system (Taylor et al., 1999a,b; Gilbertson et al., 2003; De Guise et al., 2004).

Anuran sensitivity to OP toxicity is often dependent on species, life stage, compound, and concentration (Berrill et al., 1995). Once absorbed into the body, OP compounds exert central and peripheral anticholinesterase effects, and there is

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a wide range of species variability in baseline brain AChE activity and susceptibility to anticholinesterases (Blakely and Yole, 2002). Exposure to OP insecticides can also result in morbidity and mortality via mechanisms other than anticholinesterase effects (Balasundaram and Selvarajan, 1990). Intracoelomic injection of the OP phosalone in the Indian bullfrog, *Rana tigrina*, markedly and specifically inhibits Mg^{2+}-linked ATPase enzymes of the central nervous system, precipitating a moribund (neuromuscular incoordination) or comatose state (Balasundaram et al., 1995).

In addition to neurologic effects, immunosuppression and subsequent susceptibility to opportunistic pathogens (*Aeromonas hydrophila*) has been demonstrated in Woodhouse's toads, *Bufo woodhousii*, percutaneously exposed to sublethal field doses of malathion (Taylor et al., 1999a). OP insecticides are also dose-dependent teratogens in the South African clawed frog, *Xenopus laevis* (Snawder and Chambers, 1989), and can cause eosinopenia and increased susceptibility to trematodes with consequent limb malformations at metamorphosis (Kiesecker, 2002).

Overall, anurans have been shown to be relatively resistant to acetylcholinesterase (AChE) inhibitors compared to other species (Hall and Kolbe, 1980; Wallace, 1992). Malathion aerially applied to a forested watershed at the rate of 328 g/acre caused no observed adverse effects on reptiles and amphibians, although toads adsorbed elevated loads of residues in tissues (Giles and Robert, 1970). However, birds displayed neurologic signs for 2 days, with no short-term residual neurological effects found (Giles and Robert, 1970).

Rapid and efficient detoxification pathways, leading to readily excreted compounds, are partly responsible for increased resistance to AChE inhibitors in amphibians (Wang and Murphy, 1982; Gromysz-Kalkowska and Szubartowska, 1993). For instance, frog brain acetylcholinesterase is less sensitive than acetylcholinesterase from other species studied to the inhibitory effect of organophosphorous oxons (Wang and Murphy, 1982). Woodhouse's toads percutaneously exposed to high and low doses of malathion had comparable suppression of AChE at 22% and 17%, respectively, when compared to unexposed Woodhouse's toads (Taylor et al., 1999a). Tadpoles of the *X. laevis*, exhibited significantly greater resistance to anticholinesterases compared to *X. laevis* tadpoles expressing human recombinant AChE (Shapira et al., 1998). An additional factor contributing to the resistance of amphibians to AChE inhibitors is the reduced dependence of amphibians on pulmonary respiration compared to mammals, which often succumb to respiratory paralysis, bronchoconstriction, and increased bronchial secretions (Diana et al., 2001). Because of this resistance, tadpoles can bioaccumulate certain OP pesticides to levels considered lethal to ducks and carnivorous species that consume them (Hall and Kolbe, 1980), although exposure to malathion in anurans is not without deleterious effects.

Cutaneous absorption is considered a potentially important route of environmental exposure to OP compounds for amphibians, especially in aquatic environments (Fordham et al., 2001). Of the three main routes of percutaneous xenobiotic absorption, the paracellular, transcellular, and transappendageal, the para-

cellular pathway is commonly considered the most significant in mammalian skin (Riviere, 1999). This pathway involves transport through intercellular lipids. The morphology of amphibian skin shares the distinctive "brick and mortar" morphology seen in mammalian skin (Ling, 1990). Amphibian skin possesses intercellular tight junctions, which help prevent transepidermal water loss (Tattersall and Wright, 1996) and maintain the integrity of skin, preventing or diminishing toxic, infectious, or traumatic insult.

Another route of penetration across the skin is the transcellular pathway in which molecules transfer directly through skin cells and through the intercellular lipid matrix between cells. Enhanced absorption of solutes by amphibian skin through the transcellular route, persistence of OP pesticides in aquatic environments, and the relative resistance of amphibians to cholinesterase inhibition appear to contribute to the high bioaccumulations of OP pesticides documented in amphibians (Hall and Kolbe, 1980).

The transappendageal pathway contributes to amphibian cutaneous absorption of xenobiotics through transport across the large number of cutaneous serous and mucous glands (Goniakowska-Witalinska and Kubiczek, 1998; Green, 2001). These adnexal structures could have an impact on differential cutaneous absorption of toxicants at various sites, potentially resulting in differences in absorption between anuran species. If a reservoir or depot effect for malathion occurs in the skin, periodic shedding and ingestion of sloughed skin by anurans (Green, 2001) could lead to an acute systemic toxicity via gastrointestinal absorption.

It is important to research and develop components for an amphibian model of the absorption kinetics of these compounds through the skin. Because the surface area of the entire animal is likely to be exposed in the field, the value of characterizing the skin would be beneficial in standardizing a particular anatomical site(s) for laboratory studies, in comparing species differences, and in optimizing procedures for administering medications. This model would need to evaluate skin thickness, particularly the thickness of the stratum externum, the number of cell layers, and the frequency and type of adnexal structures (Riviere and Monteiro-Riviere, 1991; Green, 2001).

In vitro studies for the characterization of cutaneous absorption can be used to model *in vivo* systems, while environmental variables, such as temperature, humidity, and pH, can be controlled. *In vitro* models also can be used to minimize the use of live animals in experiments. One *in vitro* system commonly used to determine the absorption kinetics of xenobiotics across the skin is the two-compartment Teflon flow-through diffusion cell system (Bronaugh and Stewart, 1984). Porcine skin diffusion cells have been used to extrapolate *in vivo* cutaneous exposure and metabolism of organophosphorous compounds in humans (Riviere and Chang, 1992). This system provides a morphologically complete and intact skin barrier and eliminates confounding factors in the analysis of *in vivo* cutaneous absorption, metabolism, or elimination by other routes.

We hypothesized that the two-compartment Teflon flow-through diffusion cell system will adequately model cutaneous absorption in bullfrogs (*Rana catesbeiana*) and marine toads

(*Bufo marinus*). We also hypothesized that differential skin thickness and gland distribution could result in differential cutaneous absorption between dorsal and ventral skin and between the two species.

2. Materials and methods

2.1. Husbandry

Three wild-caught bullfrogs (Charles D. Sullivan Co. Inc., Nashville, TN 37211, USA) and three marine toads (Glades Herp Inc., Bushnell, FL 33513, USA), each a minimum of 15 cm long (rostrum-to-vent) and 250 g body weight were obtained from commercial sources and acclimated for 3 weeks prior to use in the experiments. Animals were group-housed by species in 61 cm × 61 cm × 46 cm depth rectangular polyethylene tanks (ASA Industries Inc., Santa Ana, CA 92705, USA). Vivariums for bullfrogs were maintained at a 30° angle to create terrestrial, shallow, and deep water. Toads were maintained in dry level tanks with a water bowl. Complete water changes were conducted every other day using carbon-filtered water, and frog water was maintained at 21 °C using room air temperature. Fluorescent overhead lighting followed a 12 h light and 12 h dark cycle. Animals were fed to satiety daily with crickets that were allowed to engorge on rodent chow prior to being offered to the amphibians. Moistened latex and powder free gloves were used at all times when frogs and toads were handled to minimize traumatic and toxic insult to the skin.

2.2. Skin characterization

Animals were euthanized with neutral, sodium bicarbonate-buffered tricaine methanosulfonate (MS-222) at a dose of 200 mg/kg intracoelomically prior to harvesting of skin for histological examination and diffusion cell samples. Dorsal and ventral truncal skin from each of three animals of both species was preserved in Trump's fixative, embedded in paraffin, sectioned at 0.5 μm, and stained with 1% toluidine blue (Dykstra, 1993). Morphometric measurements were obtained using light microscopy (ImagePro Plus, Media Cybernetics, Silver Spring, MD 20910, USA). Thickness of the stratum externum of each sample was measured at five random points in each section (ImagePro Plus). The thickness of the stratum externum was recorded as that of the outermost layers of cornified epidermis containing pyknotic or absent nuclei (Green, 2001). The area of adnexal glands per length of basement membrane was calculated by summing the area of adnexal glands per length of basement membrane for each histological sample using a morphometry software program (ImagePro Plus). A Wilcoxon signed rank test was used for nonparametric statistical comparison of stratum externum thickness and area of adnexal glands per length of basement membrane between dorsal and ventral skin samples and between frogs and toads considering an $\alpha < 0.05$ as being statistically significant.

2.3. Absorption kinetics and tissue residues

To establish cutaneous absorption kinetics of malathion, six dorsal and six ventral full-thickness circular samples (2.8 cm²) were taken from each of three animals from both species. Samples were obtained bilaterally in two longitudinal rows of three, adjacent to the dorsal and ventral midlines. These samples were placed in two-compartment Teflon flow-through diffusion cells and perfused. The perfusate was a modified amphibian Ringer's solution (Wright, 2001) (110 mM sodium chloride, 2 mM potassium chloride, 1.3 mM calcium chloride, 2.4 mM sodium bicarbonate) spiked with bovine serum albumin (1%) and dextrose (12 g/dl) to maintain cell viability. Amikacin (62.5 mg/l) was added to limit bacterial growth, and heparin (500 U/l) was added to prevent obstruction of the perfusion lines by bovine serum albumin. The perfusate was maintained at pH 7.2–7.4, 21 °C, and a flow rate of 4 ml/h.

Malathion-2,3-^{14}C (Sigma) was diluted in 100% ethanol to achieve a dosage of 26 μg/cm² in 18.4 μl applied to each sample (0.44–0.45 μCi). Ethanol was used to enhance miscibility of malathion and achieve the desired concentration. Perfusate was collected after 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, and 360 min of malathion exposure. One millilitre of each perfusate fraction in 15 ml of EcoLume liquid scintillation cocktail (MP Biomedicals,

Irvine, CA 92618-2005, USA) was analyzed in a Packard 1900 TR Liquid Scintillation Counter (Packard Instrument Co. Inc./Canberra Industries, Meriden, CT 06450, USA).

At the end of 6 h of perfusion, the surface of the skin samples were cleaned with cotton swabs in a 1% soapy solution (Ivory dish liquid) to determine the amount of labeled malathion remaining. One side of the swab was used to apply the soapy solution, and the dry side was used for removing the solution and malathion from the skin surface. Stratum externum residues were determined by using transparent tape (six strips) to mechanically strip off the outer cornified layer. Then the dosed area of skin was removed from the peripheral region using a 0.5 cm² biopsy punch. Swabs, tapes, dosed area, and peripheral samples were separately oxidized in a Packard Model 307 Tissue Oxidizer, adsorbed with CarboSorb (Canberra Packard), mixed with 15 ml Permafluor V (Canberra Packard) scintillation fluid, and analyzed in a liquid scintillation counter. Kinetic parameters, including venous efflux profile (% dose/h), were calculated from these data. Data was examined for normal distribution by plotting residuals. The normally distributed data was examined using analysis of variance, treating different sites (dorsal and ventral) as fixed and crossed with individuals, and replicate measurements were nested within sites, individuals, and species.

3. Results

3.1. Skin characterization

Stratum externum thickness in frogs differed significantly between dorsal (19.6 ± 2.2 μm) and ventral (11.3 ± 1.7 μm) sites. This was evident in the number of cell layers in the stratum externum (Figs. 1 and 2). Dorsal skin was two to three cell layers thick, while ventral skin was one to two cells layers thick. In contrast, toad stratum externum did not differ in thickness between dorsal (15.6 ± 0.9 μm) and ventral (12.3 ± 2.8 μm) sites and consisted of two cell layers (Figs. 3 and 4). Thickness of stratum externum for dorsal skin of frogs was greater than that of toads. However, ventral skin thickness did not differ significantly between frogs and toads.

Frogs had significantly more glandular tissue in dorsal (30.8 ± 3.7 μm²/μm of basement membrane) versus ventral (11.0 ± 4.6 μm²/μm) sites (Figs. 1 and 2). The amount of glandular tissue in toad skin did not differ between dorsal (8.6 ± 4.1 μm²/μm) and ventral (17.8 ± 6.8 μm²/μm) sites (Figs. 3 and 4). In general, toad skin (dorsal and ventral) had significantly less cutaneous glandular tissue
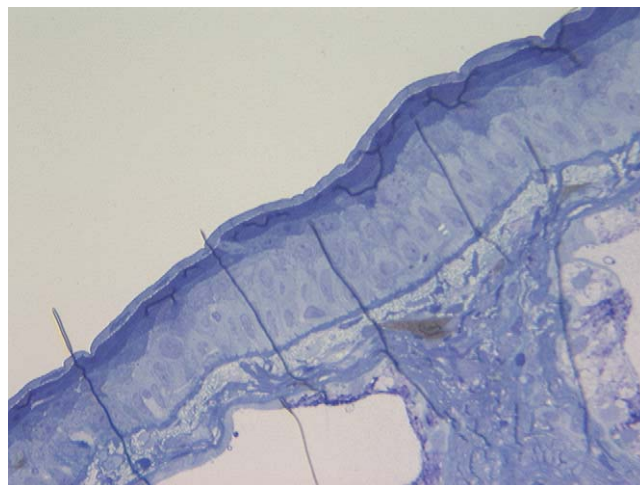

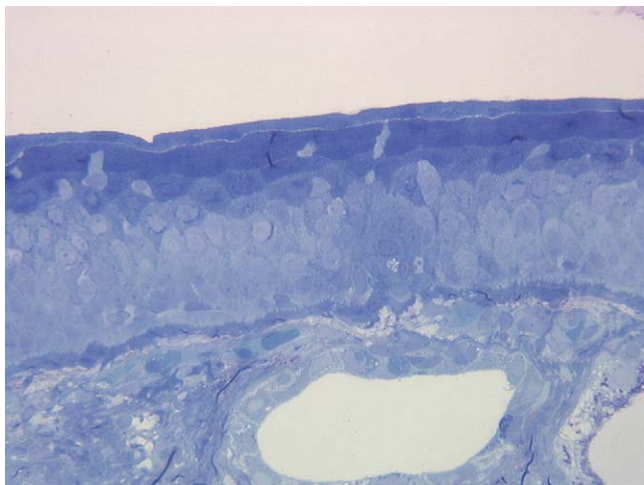Fig. 1. Frog dorsal skin, semi-thin section, toluidine blue staining, 400×.

Fig. 2. Frog ventral skin, semi-thin section, toluidine blue staining, 400×.

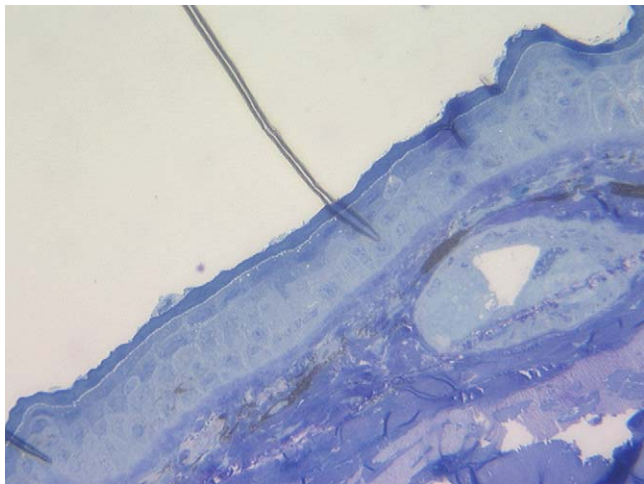

Fig. 3. Toad dorsal skin, semi-thin section, toluidine blue staining, 400×.

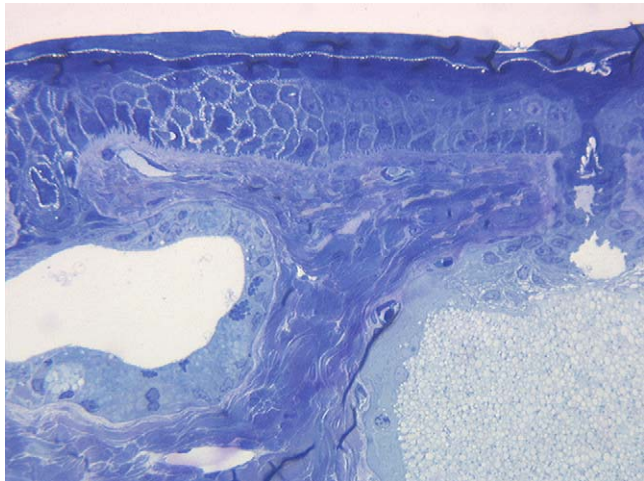Fig. 4. Toad ventral skin, semi-thin section, toluidine blue staining, 400×.

$(8.6 \pm 4.1$ and $17.8 \pm 6.8 \, \mu m^2/\mu m)$ than did dorsal skin of frogs $(30.8 \pm 3.7 \, \mu m^2/\mu m)$.

3.2. Absorption kinetics (Table 1, Figs. 5 and 6)

The total absorption (% of dose administered) for frogs was greater for ventral (81.38 ± 0.84) than for dorsal (68.51 ± 1.79) skin. Similarly, steady state flux was greater for ventral $(11.73 \pm 0.25 \, \mu g/cm^2/h)$ versus dorsal $(8.53 \pm 0.34 \, \mu g/cm^2/h)$ skin. This was reflected in an overall increase in permeability for ventral $(8.95 \pm 0.19 \, cm/h \times 10^{-3})$ versus dorsal $(6.50 \pm 0.24 \, cm/h \times 10^{-3})$ skin. Peak flux was greater for ventral $(31.44 \pm 0.73\%$ dose/h) than for dorsal skin $(22.57 \pm 0.87\%$ dose/h). However, time to peak did not vary significantly at 141.67 ± 5.18 and $135.00 \pm 4.20 \, min$, respectively.

Total absorption % for toads was also greater for ventral (82.75 ± 2.89) than for dorsal (76.77 ± 4.23) skin. However, these values could not be explained by thickness of the stratum externum because stratum externum thickness did not differ significantly between sites for toads. Time to peak also did not differ significantly between ventral $(150.83 \pm 7.71 \, min)$ and dorsal $(130.83 \pm 7.84 \, min)$ skin. Steady state flux did not differ between ventral $(11.28 \pm 0.71 \, \mu g/cm^2/h)$ and dorsal $(11.66 \pm 1.00 \, \mu g/cm^2/h)$ samples. Consequently, permeability did not differ between ventral $(8.70 \pm 0.56 \, cm/h \times 10^{-3})$ and dorsal $(9.00 \pm 0.78 \, cm/h \times 10^{-3})$ samples. Peak flux did not differ significantly between ventral $(29.76 \pm 1.90\%$ dose/h) and dorsal $(29.78 \pm 2.73\%$ dose/h) skin for toads.

Total absorption % for the dorsal skin of toads was not statistically different from that of frogs. However, steady state flux, permeability, and peak flux were significantly greater for toads than frogs. Time to peak did not differ significantly between frogs and toads for dorsal samples. There were no statistical differences between toads and frogs in any of the absorption parameters for ventral skin samples. Total recovery of radioactivity (% of administered dose) did not differ significantly for frog dorsal $(89.15 \pm 1.37\%)$, frog ventral $(94.44 \pm 0.83\%)$, toad dorsal $(91.96 \pm 2.91\%)$, or toad ventral $(95.19 \pm 0.84\%)$ diffusion cells.

3.3. Tissue residues (Table 1)

Partitioning effects in the skin were present in frogs. Swabs of the surface of the skin revealed a significantly greater retention of malathion on the dorsal skin surface $(0.63 \pm 0.11\%$ dose administered) than the ventral skin surface $(0.32 \pm 0.06\%$ dose administered) of frogs. Retention of malathion in the stratum externum was also significantly greater in frog dorsal skin $(0.69 \pm 0.06\%$ dose administered) than in frog ventral skin $(0.43 \pm 0.03\%$ dose administered). Skin retention was also reflected by greater diffusivity of dorsal $(527.14 \pm 42.98 \, cm^2/h \times 10^{-6})$ versus ventral skin $(429.10 \pm 19.11 \, cm^2/h \times 10^{-6})$. The layers beneath the stratum externum in dorsal frog skin retained more of the % dose administered dose $(5.26 \pm 0.45\%)$ than they did in ventral frog skin samples $(2.31 \pm 0.18\%)$.

In contrast, partitioning effects for malathion were not present in toad skin. Malathion values for swabs were $0.59 \pm 0.09\%$
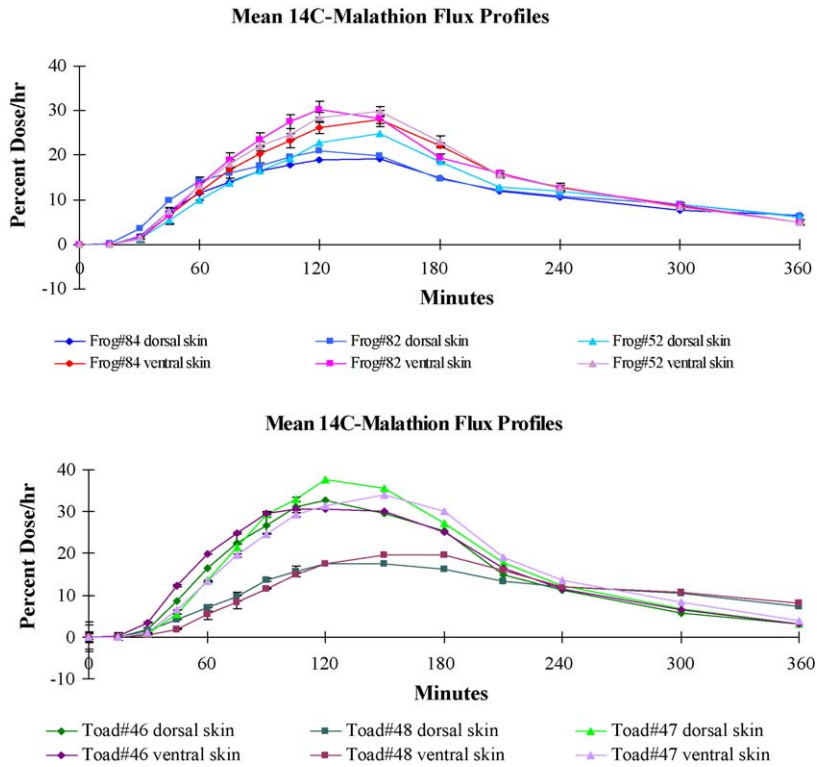
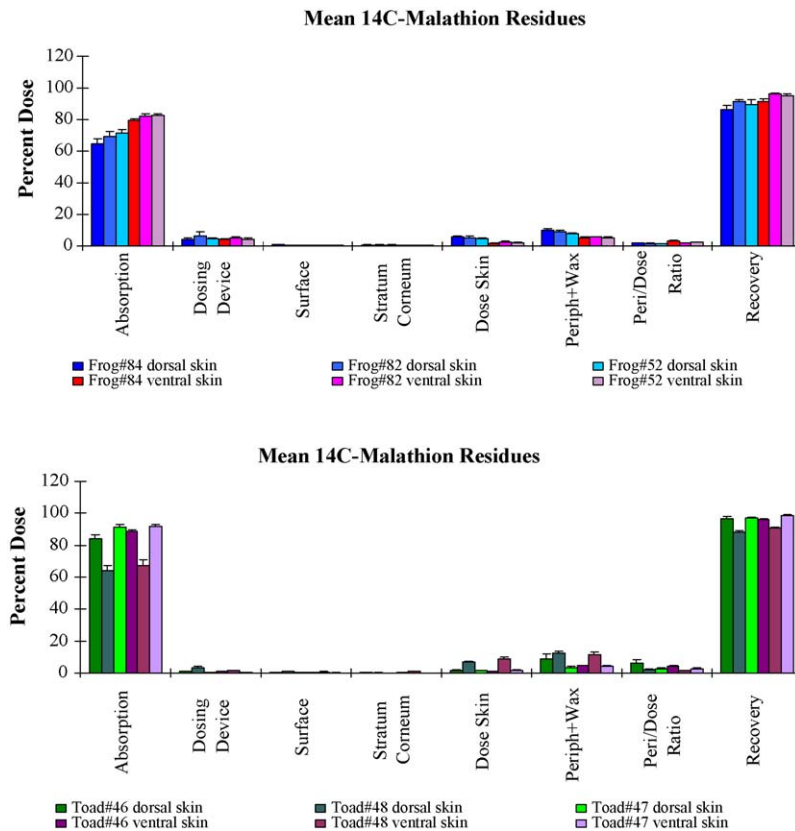Fig. 5. Mean ($n = 6$) and S.E. malathion flux profiles represented as flux (% of 26 μg/cm^2 dose/h) vs. time.

Fig. 6. Mean ($n = 6$) and S.E. malathion residues represented as % dose (26 μg/cm^2) absorbed, dosing device (diffusion cell), surface (swabs), stratum externum/corneum (tape strips), dosed skin, peripheral tissue and wax paper, peripheral/dosed skin ratio, total recovery.

Table 1
Mean absorption kinetic parameters ($26\,\mu g/cm^2$ dose)

Animal	Frog dorsal mean	Frog ventral mean	Toad dorsal mean	Toad ventral mean
Absorption (% of dose)	68.51	81.38	76.77	82.75
Dosing device (% of dose)	5.08	4.52	1.53	0.95
Swab (% of dose)	0.63	0.32	0.59	0.45
Tape (% of dose)	0.69	0.43	0.29	0.43
Dosed skin (% of dose)	5.26	2.31	3.22	3.8
Peri + wax (% of dose)	8.98	5.48	8.55	6.81
Recovery (% of dose)	89.15	94.44	91.96	95.19
Peak flux (% of dose)	22.57	31.44	29.78	29.76
Time to peak (min)	135	141.67	130.83	150.83
Steady-state flux ($\mu g/cm^2/h$)	8.53	11.73	11.66	11.28
Permeability ($cm/h \times 10^{-3}$)	6.5	8.95	9	8.7
Diffusivity ($cm^2/h \times 10^{-6}$)	527.14	429.1	443.23	429.92

and $0.45 \pm 0.07\%$ of the absorbed dose for dorsal and ventral toad skin, respectively. Retention of malathion in the stratum externum was $0.29 \pm 0.04\%$ and $0.43 \pm 0.10\%$ of the absorbed dose for dorsal and ventral toad skin, respectively. The % dose of malathion administered retained in the dosed area of skin beneath the stratum externum in toads did not differ between dorsal ($3.22 \pm 0.67\%$) and ventral ($3.80 \pm 0.96\%$) skin samples. Consequently, diffusivity was not significantly different when comparing between dorsal ($464.70 \pm 33.11\,cm^2/h \times 10^{-6}$) and ventral ($439.92 \pm 30.11\,cm^2/h \times 10^{-6}$) skin samples.

There were, however, some statistically significant differences in partitioning effects in dorsal skin between frogs and toads. Malathion retention in the stratum externum and dosed area beneath the stratum externum were greater for frogs than for toads. However, neither diffusivity across dorsal skin nor retention on the surface of the dorsal skin differed between the two species.

4. Discussion

Decreased absorption and flux in the dorsal skin of frogs compared with the ventral skin can be attributed to several anatomical and physiological variations. The increased thickness of the stratum externum of the dorsal skin is evident in the increased number of cell layers, as well as overall thickness. This outermost cornified layer of skin is the greatest source of resistance to percutaneous absorption and subsequent vascular uptake by capillaries in the dermis (Riviere, 1999). The thickness of the stratum externum is also a major determinant of paracellular and transcellular routes of absorption (Riviere, 1999). These factors could explain why stratum externum (tape strip) residues were greater in dorsal frog skin, and permeability was decreased.

The increased presence of mucous and serous glands in the dermis of the dorsal skin of frogs could create a depot effect for malathion, further impeding vascular uptake. At the same time, these glands could also enhance absorption into the skin through the transappendageal route resulting in the dosed area of dorsal skin (sub-stratum externum and dermis) containing higher residues of malathion than ventral skin. This could also explain an increased diffusivity in the dorsal skin. The secretions

of the glands to the surface of the skin may create an additional barrier to absorption, and/or create a depot effect, which could explain higher dorsal skin surface (swab) residues for frogs.

Significantly more malathion was retained on the surface of the skin, in the stratum externum, and in the deeper layers of skin for dorsal samples in frogs. Therefore, less malathion was absorbed. Redistribution of malathion from the deep compartment (depots), ingestion of sloughed skin, and the potential for whole body exposure could reduce the practical impact of the differences between dorsal and ventral absorption. In contrast, frogs directly exposed to aerosolized applications of malathion would likely receive dorsal exposure preferentially, and therefore, the lower permeability of the dorsal skin might afford some measure of protection.

Malathion is applied to crops and the environment in the form of aerosolized droplets approximately the same size as the $18.4\,\mu l$ dose used in this study (Perich et al., 2000), which should not be sufficiently small ($1–5\,\mu g$) to reach the lower airways for respiratory absorption (Riviere, 1999). Typically, malathion aerosols are applied to the environment at a rate of $100\,g/acre$ ($2.5\,\mu g/cm^2$), significantly less than the $26\,\mu g/cm^2$ local application in the diffusion cell study (Perich et al., 2000). A percutaneous sublethal dose of $11\,\mu g/g$ of body weight has been demonstrated for bullfrogs and marine toads (Taylor et al., 2004). A frog or toad exposed dorsally to a typical environmental aerosol application of malathion would be expected to be exposed to sublethal doses or greater in favorable weather conditions.

In contrast to frog skin, the absence of significant differential flux, permeability, and diffusivity between dorsal and ventral skin of toads could potentially be explained by the lack of differential thickness of stratum externum and gland distribution in toads. However, differences in kinetic parameters between the dorsal skin of frogs and all skin samples from toads can be attributed to the same anatomical differences present between the dorsal and ventral skin of frogs. Toad skin had a thinner stratum externum and contained fewer mucous and serous glands than the dorsal skin of frogs. The physiochemical properties of the components of glandular secretions also could impact the percutaneous absorption or depot retention of malathion.

There was greater variability in the percutaneous absorption kinetic profiles between individual toads compared to individual frogs. Considering an $n = 3$, it is possible that the kinetic profile of toad 48 is more representative of toads than the other two. Alternatively, variation in permeability could be the norm, which could impart a selective advantage of malathion tolerance to toad 48.

The diffusion cells in our study do not completely replicate natural exposure conditions. For example, to avoid miscibility challenges in the small dosing units we were applying, we used pure ethanol as the vehicle for the applied malathion. Practical spray applications use a variety of vehicle solvent combinations depending upon the application (Marty et al., 1994). These solvent mixtures could be expected to affect the kinetics observed in the application. However, in most environmental exposures skin is more likely to be exposed to an aqueous or oily formulation than to pure solvent (Horstman et al., 1989). Therefore, true environmental exposure risk may be higher than in our studies because malathion is more likely to partition to the skin from an aqueous or oily formulation. This model could be strengthened by exploring other vehicles (field exposure dilution in water), different applied concentrations, and combination with polar penetrants, rubifacients, or detergents. Integrity of the skin can also be determined by tritiated water flux.

Two-compartment Teflon flow-through diffusion cells were useful to determine absorption kinetics of malathion in amphibian skin. Accounting of total radioactive dose for both sites and species indicated excellent recovery and consistency. Understanding the percutaneous absorption in amphibians is instrumental in assessing exposure to xenobiotics such as malathion and determining resulting impacts on animal health and the environment. Anurans are sentinel species for environmental contamination of OP pesticides such as malathion, and percutaneous absorption represents a significant route of exposure. Topical exposure to general agricultural and environmental applications of sublethal doses of malathion could result in detrimental neurologic and immunologic effects.

Acknowledgements

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